An Open-source IVIVE Workflow Integrating In Vitro Data, QSAR Models, and Reverse Dosimetry

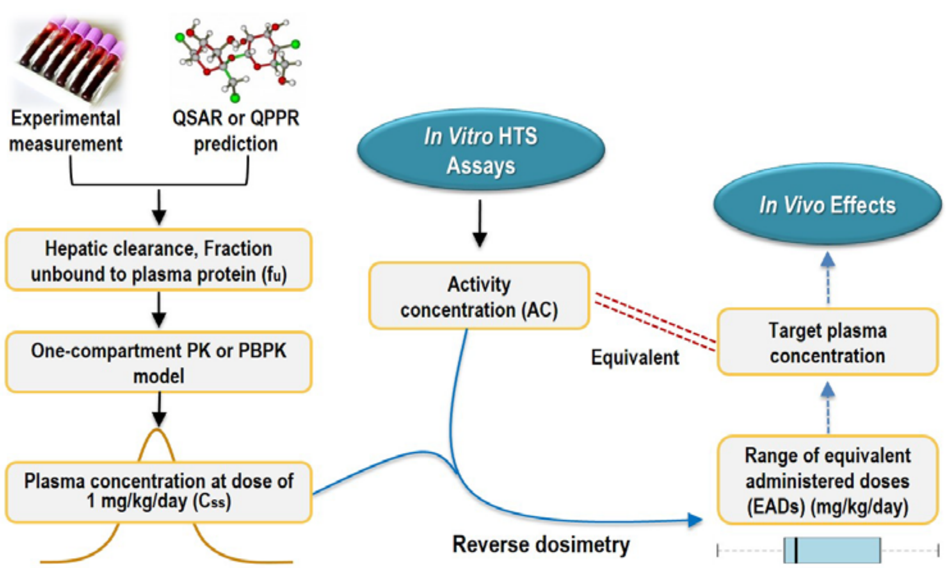
X. Chang[1], S. Bell[1], R. Rai[1], J. Phillips[2], K. Mansouri[1], D. Allen[1], W. Casey[3], N. Kleinstreuer[3]

[1]*ILS, RTP, NC, USA;* [2]*Sciome LLC, RTP, NC, USA;* [3]*NIH/NIEHS/DNTP/NICEATM, RTP, NC, USA*

Introduction

- A critical challenge to implementing non-animal approaches for chemical safety testing is linking in vitro assay results to potential in vivo effects.
- In vitro to in vivo extrapolation (IVIVE) predicts the daily in vivo equivalent administered dose that would result in a plasma concentration corresponding to an in vitro effective concentration (**Figure 1**).
- To facilitate IVIVE analysis, we developed an open-source IVIVE workflow incorporating in vitro assay data, quantitative structure-activity relationship (QSAR) models, a quantitative property-property relationship (QPPR) model, and reverse dosimetry.

Figure 1 A Reverse Pharmacokinetic Model for IVIVE



Open-source IVIVE Workflow

- The IVIVE workflow is publicly accessible through the Integrated Chemical Environment (ICE) web resource (https://ice.ntp.niehs.nih.gov/; Bell et al. 2017) developed by the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

- ICE houses:
  - Curated in vitro and in vivo assay data that cover a range of toxicological endpoints
  - In silico predictions of a chemical's biophysical and biochemical properties
  - Computational tools and workflows
- The IVIVE workflow can be run as an interactive online tool via the ICE graphical user interface (**Figure 2**) or downloaded as an R notebook to run locally. **Figure 3** shows the scheme of the workflow.
- Required workflow inputs include:
  - In vitro assay activity provided via ICE and selectable by user; activity is expressed as either:
    - Half-maximal activity concentration (AC_{50})
    - Activity concentration at cutoff (ACC)
  - Pharmacokinetic (PK) parameters, provided by user or via ICE using experimental data or published in silico models (Kirman et al. 2015; Mansouri et al. 2018):
    - Fraction of chemical unbound to protein
    - Intrinsic clearance
    - Renal clearance
- A population-based PK model is used for reverse dosimetry, and estimates steady-state plasma concentration (C_{ss}) following a given dose for a Monte Carlo simulated population, covering physical variability across individuals (Wetmore et al. 2012) (**Figure 4**).
- The workflow output is the daily equivalent administered dose (EAD) that would lead to the C_{ss} equivalent to the AC_{50} or ACC from the in vitro assay of interest. The user has two options for calculating the EAD:
  - EAD corresponding to total chemical concentration:

$$EAD = ACC(or\,AC50) \times \frac{1}{C_{ss}} (mg/kg/day)$$

  - EAD corresponding to unbound chemical concentration (default option):

$$EAD_{f_u} = EAD \times \frac{1}{f_u} (mg/kg/day)$$

- The EAD output values can be compared to in vivo lowest effective levels (LELs) if data are available.

Figure 2 ICE User Interface

Figure 3 IVIVE Workflow Overview

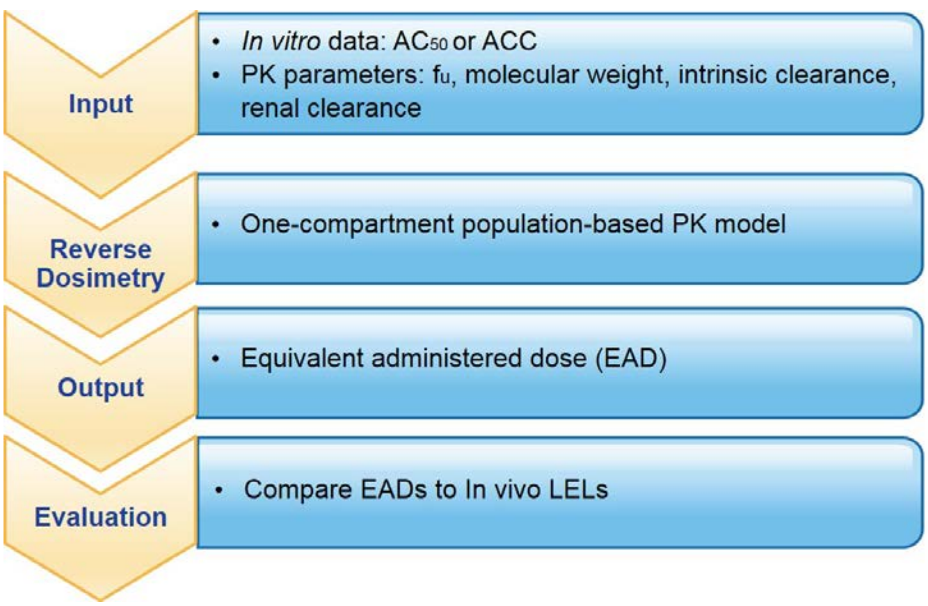
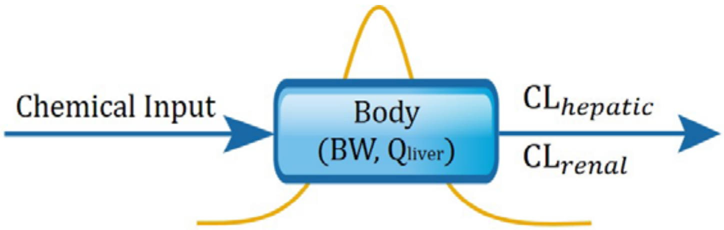


Figure 4 One-compartment Population-based PK Model



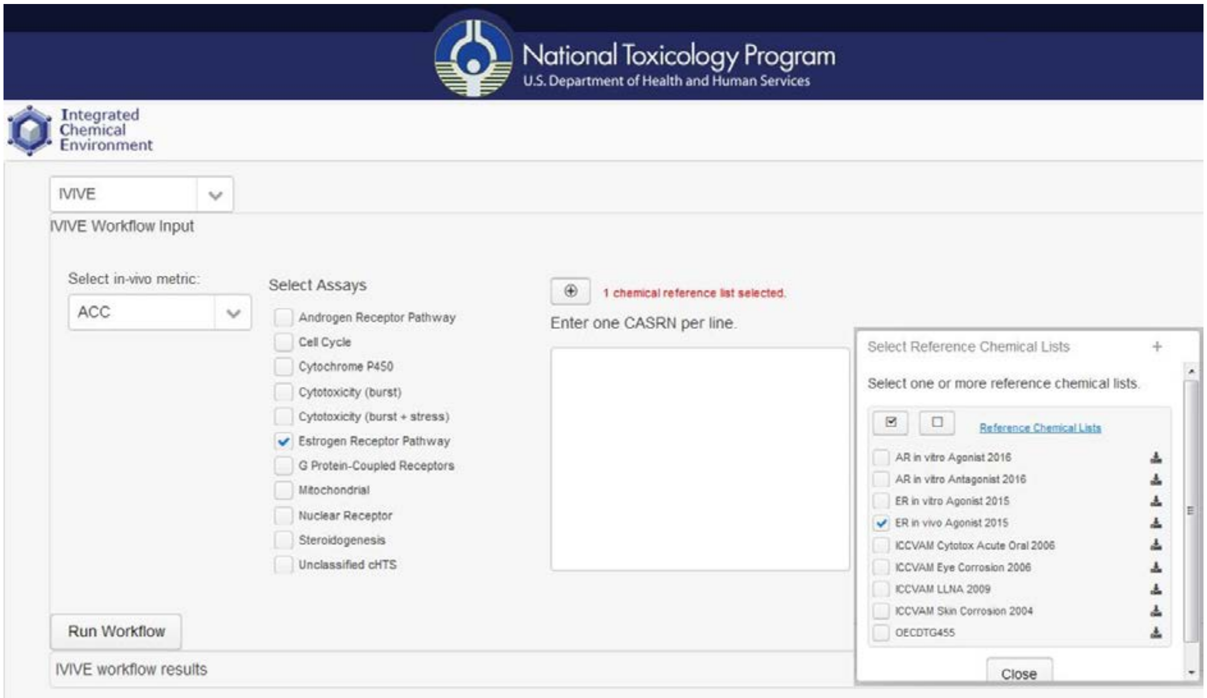
$$C_{ss} = \frac{\text{Standard dose rate (1 mg/kg/day)*BW}}{\underbrace{(\text{GFR} \times f_u)}_{\text{CL}_{renal}\text{(L/h)}} + \underbrace{\left(\text{Q}_{\text{liver}} \times \frac{f_u \times CL_{\text{int}}}{\text{Q}_{\text{liver}} + f_u \times CL_{\text{int}}}\right)}_{\text{CL}_{hepatic}\text{(L/h)}}} \times \frac{1000}{MW \times 24}$$

BW=body weight; CL=clearance; GFR=glomerular filtration rate; MW=molecular weight; Q=blood flow rate

Workflow Application: ER Pathway Assays

- We used ACC values from 39 estrogen receptor (ER) reference chemicals tested in 18 ER pathway assays as inputs to the workflow (**Figure 5**).
- **Figure 6** shows the workflow output. For this example, we compared the EAD_{fu} values obtained from the workflow with LELs from in vivo injection uterotrophic assays (Kleinstreuer et al. 2016; Casey et al. 2018; **Table 1**).

Figure 5 Screenshot of ICE IVIVE Workflow Input



The figure shows an example of selecting workflow inputs, including in vitro metric, group of assays, and chemicals. For this example, we used the ACC, ER assays, and ER in vivo agonist reference chemical list.

Figure 6 Screenshot of ICE IVIVE Workflow Output

The figure shows the EAD (mg/kg/day; blue boxplots) predicted from ACC (µM; orange boxplots) of ER pathway assays using the ICE IVIVE workflow.

Table 1 Comparison of ICE Workflow Output with In Vivo Data

CASRN	Chemical Name	EAD_{fu} (ICE Output)	Lowest LEL_{inj}	Median LEL_{inj}	Highest LEL_{inj}	Number of studies
72-43-5	Methoxychlor	131.38	50	100	100	12
56-53-1	Diethylstilbestrol	0.078	5E-05	0.00025	0.002	5
521-18-6	5alpha-Dihydrotestosterone	102.12	4	20	200	3
446-72-0	Genistein	3.22	1	15	35	19
77-40-7	Bisphenol B	5.70	20	110	200	2
98-54-4	4-tert-Butylphenol	314.91	99.2	99.6	100	2
104-43-8	4-Dodecylphenol	15.89	40	40	40	3
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	53.60	200	200	200	2
17924-92-4	Zearalenone	0.50	2	2	20	3
5153-25-3	2-Ethylhexylparaben	43.77	200	200	200	2
474-86-2	Equilin	0.077	2	2	2	2
53-16-7	Estrone	0.024	0.0018	0.102	2	6
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	18.29	200	200	300	5
68-22-4	Norethindrone	14.30	2	11	20	2
72-33-3	Mestranol	1.44	0.0016	0.0038	0.006	2
599-64-4	4-Cumylphenol	31.84	20	110	200	2
1478-61-1	Bisphenol AF	3.39	4	6	8	4
57-63-6	17alpha-Ethinylestradiol	0.012	0.0001	0.0003	0.002	35
131-56-6	2,4-Dihydroxybenzophenone	108.51	100	200	300	2
104-40-5	4-Nonylphenol	235.13	100	200	200	3
94-26-8	Butylparaben	69.25	50	300	1000	8
789-02-6	o,p'-DDT	31.94	1	100	200	9
58-18-4	17-Methyltestosterone	116.30	10	10	10	3
80-05-7	Bisphenol A	12.17	2	100	800	29
80-09-1	4,4'-Sulfonyldiphenol	180.35	20	20	20	2
80-46-6	4-(2-Methylbutan-2-yl)phenol	45.30	200	200	202	4
50-28-2	17beta-Estradiol	0.017	0.0001	0.001	2	19

Discussion and Conclusion

- The ICE IVIVE workflow provides an open-source, easy-to-use tool for IVIVE analysis.
- The workflow can be used to evaluate the correlation between in vitro and in vivo activity for toxicologically relevant end points.
- For chemicals lacking in vivo data, it can be used to predict relevant toxicity potential, expediting the safety assessment process.
- When evaluating estrogenic activities, the range of EAD estimates produced by the workflow correlated well with the range of in vivo uterotrophic LELs for the chemicals tested, suggesting the IVIVE approach provides valid estimates of in vivo estrogenic activity from in vitro ER pathway assays.

References

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Mansouri et al. 2018. J Cheminform 10(1):10.

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